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(54) Title: ENHANCED UPTAKE DRUG DELIVERY SYSTEM

(57) Abstract

A drug delivery system including a plurality of microsphere particles containing an active drug and including a surfactant material associated with each particle which surfactant material has the property of enhancing the uptake of the active drug.

ENHANCED UPTAKE DRUG DELIVERY SYSTEM

FIELD OF THE INVENTION

The present invention relates to drug delivery systems and more particularly to a system which enhances the uptake of active drug material, particularly high molecular weight materials, especially from the nasal cavity.

References will be made to technical papers and other disclosures within this field which are included for purposes of explanation.

European patent applications 023,359 and 122,023 describe a powdery pharmaceutical composition for application to the nasal mucosa and methods for administration thereof. The pharmaceutical composition allows polypeptides and derivatives thereof to be effectively absorbed through the nasal mucosa. Similarly US Patent 4250163 describes a method for administering a medicament to the nasal mucosa where the preferred composition has mucoadhesive properties. European Patent application 123,831 has described how the use of bio-compatible water soluble amphiphilic steroids other than natural bile salts are capable of increasing drug permeability across body surfaces to include the nose. The German Patent 2620446 describes an aqueous insulin preparation for nasal application containing a penetration enhancer in the form of an amphoteric, anionic or nonionic surface active agents, saponin, bile salts or surfactin. European patent application 230,264 describes an aqueous nasal drug delivery system for vaccines containing a high molecular weight drug, a gelling agent (e.g. hydroxy-ethylcellulose) and in some cases other additives (e.g. surfactants, glycerol, polyethyleneglycol).

absorbed effectively with a bioavailability similar to that for an intravenous injection; the peak concentration appearing after approximately 6 minutes. If progesterone is given via the oral route then published data suggest that the bioavailability is of the order of 1.2% as compared to IV administration (1). The second example is the beta-blocker propranolol. This drug is metabolised extensively in the liver and possibly in the gut wall when administered orally. When the drug is given intranasally in a simple solution, plasma levels identical to intravenous administration can be obtained (2).

Insulin, a drug that has been studied extensively for intranasal delivery, can be delivered across the nasal membrane but the absorption efficiency is normally about 5% of the adminstered dose. Absorption can be improved by the use of so-called absorption enhancers. For example in a study by Salzman insulin was administered in the presence of a surfactant, Laureth 9 (3). Not only was a clear dose response relationship obtained but also the peak level appeared rapidly. The potency of the intranasal insulin was approximately 1/10th that of intravenous administered insulin. Clearly if insulin can be delivered to patients in a safe and reliable way by nasal administration then such systems could have potential for administration with meals in type 1 diabetes.

Chien and Chang (4) have summarised the absorptive capacity of the nasal routes for a variety of drug substances. It will be noted that those materials of high molecular weight, i.e. peptides and proteins are normally poorly absorbed via the nasal route. Also is noted the fact that most of the compounds, both those with high and low absorption efficiencies, show peak plasma levels within approximately 30 minutes. Thus absorption, whatever its extent, appears to be rapid but does not

selected included albumin, starch and the ion exchange material DEAE-Sephadex. the size of the microspheres has been of the order of 40-60 um in diameter.

The clearance of labelled microspheres from the nose has been studied in human volunteers using the standard technique of gamma scintigraphy (7). The microspheres were labelled with technetium-99m and applied to the nose in powder form using a nasal insufflator. Liquid and powder formulations were used as controls. The position of the noses of the volunteers was held constant on the collimator of the gamma camera using a specially designed template. Scintiscans were obtained over a suitable time period and regions of interest were created around the site of deposition in the nasal cavity. The time-activity profiles showed clearly that the nasal spray and powder formulations are cleared quite rapidly (with a time for 50% clearance ($T_{50\%}$) of 15 minutes). In contrast, the microsphere systems have a much longer clearance time. After 3 hours about 50% of the albumin and starch microspheres and 60% of the DEAE-Sephadex microspheres still remain at the site of application. The half-time of clearance from this initial deposition site for DEAE-Sephadex microspheres were calculated to be about 4 hours. At the present time we are exploring whether these microsphere systems will provide an enhancement of the bioavailability of selected drug substances to include peptides and proteins. We expect that a decreased clearance rate and the possible protection of labile drugs against enzymatic attack will significantly increase absorption efficiency.

In relation to controlled release systems and the nose it is interesting to note that Nagai and colleagues (8) have been able to increase the absorption of insulin after nasal application to dogs by using a gelling formulation. Insulin was mixed with a cellulosic material and Carbopol 934 (polyacrylic acid) and applied as a

surfactant material associated with each particle, which surfactant material has the property of enhancing the uptake of the active drug.

Preferably the particles are administered in the form of a powder by spraying and have bioadhesive properties.

The surfactant material should not produce any problems in terms of chronic toxicity because in vivo the surfactant should be non-irritant and/or rapidly metabolished to a normal cell constituent that does not have any significant irritant effect. A preferred surfactant material is lysolecithin and other lysophosphatidyl compounds such as lysophosphatidyl-ethanolamine, lysophosphatidic acid ect. A suitable concentration is from 0.02 to 10%.

Embodiments of the present invention will now be described, by way of example with reference to the accompanying drawings, in which:

Figure 1 illustrates in graphical form the effect of the use of the natural surfactant material on the uptake of a drug in a first experiment;

Figure 2 illustrates in graphical form the effect of the use of the natural surfactant material and the administration in the form of microspheres;

Figure 3 illustrates in graphical form the effect of the use of the natural surfactant in a rat study experiment;

Figures 4 and 5 respectively show plasma glucose levels for rabbits given intranasal doses of Zn and Na -insulin;

Figure 6 shows plasma glucose levels obtained for administration intranasally of insulin in different forms;

Figure 7 shows corresponding curves for plasma insulin levels;

In some instances the longer contact time alone can provide a satisfactory improvement in biological availability.

A preferred enhancing material is the material lysophosphatidylcholine produced from egg or soy lecithin. Other lysophosphatidylcholines that have different acyl groups as well as lyso compounds produced from phosphatidylethanolamines and phosphatidic acid which have similar membrane modifying properties may be used. Acyl carnitines (e.g. Palmitoyl-DL Canitine-chloride) is an alternative.

Other enhancing agents that would be appropriate for the present invention include chelating agents (EGTA, EDTA, alginates), surface active agents (especially non-ionic materials), acyl glycerols, fatty acids and salts, tyloxapol and biological detergents listed in the SIGMA Catalog, 1988, page 316-321. Also agents that modify the membrane fluidity and permeability would be appropriate such as Enamines (e.g. phenylalanine enamine of ethyl-lacetoacetate), Malonates (e.g. diethyleneoxymethylene malonate), Salicylates, Bile salts and analogues and fusidates. Suitable concentrations would be up to 10%.

The same concept of delivery of a drug incorporated into or onto a biadhesive microsphere with an added pharmaceutical adjuvant would apply to systems that contained active drug and mucolytic agent, peptidase inhibitors or irrelevant polypeptide substrate singly or in combination. A suitably mucolytic would be thiol containing compounds such as N-acetylcysteine and derivatives thereof. Peptide inhibitors include Actinonin, Amastatin, Antipain, Bestatin, Chloroacetyl-HOLeu-Ala-Gly-NH2, Diprotin A and B, Ebelactone A and B, E-64, Leupeptin, Pepstatin A, Phisphoramidon, H-Thr-(tBu)-Phe-Pro-Oh, Aprotinin, Kallikrein Inh.1,

very much greater than the enhancement that can be achieved by the enhancer itself. This potentiation of enhancer action is believed to be due to the greater retention of the delivery system in the nasal cavity. The concept has been shown to be successful for different drugs such as gentamicin, insulin and growth hormone. The enhancer selected for these studies has been lysophosphatidylcholine (described above). The concept should work equally well with other enhancer system (see list elsewhere) and with other drugs such as:

Insulin (hexameric/dimeric/monomeric forms)
Glucagon
Growth Hormone (Somatotropin)
Calcitonins and synthetic modifications thereof
Enkephalins
Interferons (especially Alpha-2 Interferon for treatment of common colds)
LHRH and analogues (Nafarelin, Buserelin, Zolidex)
GHRH (Growth hormone releasing hormone)
Secretin
Nifedipine
Bradykin antagonists
GRF (Growth releasing factor)
THF
TRH (Thyrotropin releasing hormone)
ACTH analogues
IGF (Insulin like growth factors)
CGRP (Calcitonin gene related peptide)
Atrial Natriuretic Peptide
Vasopressin and analouges (DDAVP, Lypressin)
Antibiotics
Metoclopramide
Migraine treatment (Dihydroergotamine, Ergometrine, Ergotamine, Pizotizin)
Nasal Vaccines (Particularly AIDS vaccines)
FACTOR VIII

System). Details of other devices can be found in the pharmaceutical literature (see for example Bell, A. Intranasal Delivery devices, in Drug Delivery Devices Fundamentals and Applications, Tyle P. (ed), Dekker, New York, 1988).

Animal nasal delivery studies

The following studies of nasal delivery in animal models (rats, rabbits and sheep) has been carried out in order to substantiate the invention.

Gentamicin:

The drug gentamicin was chosen as a model test substance. This polar compound is known to be poorly absorbed when administered into the nose (see for example Duchateau et al (17) and the biological availability can be enhanced by added bile salts.

Rat Studies:

The in situ rat model of Hirai et al (14) was used as modified by Fisher et al (15). Male Wistar rats of about 200 g were anaesthesized by intraperitoneal injection of 60 mg/kg of Pentobarbitone (Sagatal, 60 mg/ml). The rats were tracheotomized, the oesophagus sealed and the carotid artery cannulated.

A volume of the gentamicin solution containing 0.5% of the drug with and without added lysophosphatidylcholine (LPC) (0.2%) was instilled into the nasal cavity. Blood samples were withdrawn from the carotid artery at 0, 5, 10, 15, 30, 45, 60 and 120 min after drug administration. The gentamicin level was determined by the EMIT method (16). The effect of the LPC enhancer is demonstrated in Figure 1. The administration of gentamicin solution alone resulted in a poor bioavailability whereas the adding of the enhancer system gave rise to a five fold greater peak level. The AUC's (from t=0 to t=120 min) were 128 and 557 ug min/ml, respectively.

as a solution (40 mg/ml) through the jugular vein. Blood samples (2 ml) were collected through the jugular vein at 0, 8, 16, 24, 32, 45, 60, 90, 120, 180 and 240 min after drug administration. The serum was separated by centrifugation and the samples stored at -20°C awaiting analysis. No heparin was added to any of the samples. The gentamicin level was determined by the EMIT technique (16).

A dramatic effect is seen when the gentamicin plus enhancer are adminstered in the form of the starch microsphere formulation, the blood level peaking at 6.3 ug/ml as compared to 0.4 ug/ml for gentamicin solution. The combination of microspheres plus LPC enhancer provides a blood level-time profile that is very similar to that obtained when gentamicin is given intravenously (Figure 2).

Accounting for the doses administered the bioavailability for the intranasally adminstered gentamicin in combination with the LPC enhancer and gelling microsphere system is 57.3% as compared to the gentamicin given by IV dose.

Insulin

In all animal studies the glucose plasma levels were analysed using the glucose oxidase method. The plasma insulin levels were determined for the rabbit and sheep experiments by means of radioimmune assay using a double-antibody technique.

Rat Studies:

The Hirai's in situ model (as modified by Fisher) was used to study the nasal absorption of insulin using non-diabetic male Wistar Rats of 150 g fasted overnight. The rats were anaesthesized with an i.p. injection of 0.25 ml of Pentobarbitone (60 mg/ml).

A 250 IV/ml solution of Zinc (Zn)-human insulin was prepared in buffer (1/75 M Na_2HPO_4) of pH 7.3. In some experiments 0.2% of LPC or for comparison 1% Glyco-

The rabbits were dosed s.c. with insulin at 0.8 IU/kg or 0.6 IU/kg from a 14 IU/ml or 10 IU/ml aqueous solution, respectively.

The dose of starch microspheres and insulin was fixed at 2.5 mg/kg and 2.5 IU/kg, respectively. The dose of LPC was 0.2 mg/kg. The average weight of the rabbits was 3.5 kg.

25 mg of microspheres were placed in a small glass vial and 250 ul of a 100 IU/ml insulin solution (Na- or Zn- insulin) was added followed by the 2 mg LPC and 250 ul of distilled water. The microspheres were then allowed to stand for 2h at room temperature in contact with the insulin solution before freeze drying.

Approximately 15 mg of the freeze dried powder from each individual vial was filled into the applicator tubing, and this was stored in a dessicator until use.

The rabbits were administered the suggested dose into the nasal cavity without sedation. Each rabbit was held on its back during, and for 10 second after, the application to ensure the delivery of the powdered formulation. Blood samples of 200 ul and 2 ml for glucose and insulin determination, respectively, were collected from the marginal ear vein at 10 and 5 min prior to the administration and at 5, 15, 30, 45, 60, 90, 120 and 180 min post-administration. For insulin analysis, the blood collected was mixed gently in 5 ml heparinised (Li Heparin) tubes. For glucose analysis, the blood collected was mixed gently in 5 ml fluoride oxalate tubes. The blood samples for glucose analysis were kept on crushed ice awaiting immediate analysis. The blood samples for insulin analysis were spun at 3000 rpm and the plasma collected was stored at -20°C awaiting analysis.

Figures 4 and 5 show the plasma glucose levels for rabbits given intranasal doses of Zn-insulin or Na-insulin, respectively as simple solutions, in simple solutions with 0.2% LPC added or in combination with

Preparation of insulin solutions and powders:

Insulin stock solutions were prepared in 1/75 M phosphate buffer (pH 7.3). These were then used as liquid formulations for intravenous and intranasal administration, and also in the preparation of the lyophilised microsphere formulations. The latter were prepared by dispersing the required quantity of microspheres in the insulin solution (+ any LPC), stirring for 1 hour at room temperature, and then freeze-drying to obtain the powder formulation.

Administration of insulin formulations:

Insulin was administered at 0.1 IU/kg via the intravenous route, at 0.2 IU/kg via the subcutaneous route, and at 2 IU/kg via the nasal route. Three sheep were used in each experiment:

(1) Intravenous administration of insulin as an aqueous solution prepared at 4IU/ml : Sheep J, K, and L on 24/11/87.

(2) Intranasal administration of an aqueous solution, prepared at 200 IU/ml : Sheep A, B, and C on 24/11/87.

(3) Intranasal administration of an aqueous solution, prepared at 200 IU/ml in combination with 0.2% LPC (0.02 mg/kg) : Sheep D, E, and F on 24/11/87.

(4) Intranasal administration of insulin in combination with starch microspheres (2.5 mg/kg) and LPC (0.20 mg/kg) as a lyophilised powder. To prepare the formulation 500mg of Spherex were dispersed in 30 ml of 1/75M phosphate buffer (pH 7.3) containing 400 IU insulin and 40 mg LPC, mixed for 1h, and then freeze-dried : Sheep M, N and O on 26/11/87.

(5) Intranasal administration of starch microspheres (2.5 mg/kg) without insulin. To prepare the formulation, 500 mg of Spherex were dispersed in 30 ml of 1/75M phosphate buffer (pH 7.3), mixed for 1h, and then freeze-dried : Sheep G, H, and I on 24/11/87.

Figure 6 shows the plasma glucose levels obtained for the administration intranasally of a simple insulin solution, of blank starch microspheres, of insulin solution with added 0.2% LPC, insulin as a microsphere formulation in combination with LPC and the intravenous administration of insulin. Figure 7 shows the corresponding curves for plasma insulin levels. As seen in the rat and rabbit studies insulin administered intranasally as a simple solution does not have a significant effect on the plasma glucose level and the amount of insulin being absorbed via this route is indeed very low. Adding the enhancer system (LPC) to the formulation increases the amount of insulin appearing in the circulation and hence results in a somewhat lower plasma glucose level. The administration of the insulin in combination the starch microspheres and LPC results in a 693% increase in AUC of plasma insulin as compared to a simple nasal insulin solution. At the same time the peak insulin level is increased with 1040%. The sharp level peak appears at 15-20 min and decreases rapdily as for intravenous insulin. Considering the glucose levels obtained when administering the insulin-microsphere-enhancer system the shape of the plasma glucose profile is very similar to the one obtained for the intravenous insulin. The relative bioavailability for this sytem is about 25% as compared to a subcutaneous injection of insulin.

Human growth hormone

For all experiments biosynthetic hGH was used. The plasma levels were analysed using a solid-phase 2-site sandwich-ELISA technique. Plasma was assayed in duplicate at a dilution of 1/10 against a standard solution of B-hGH (0.11-7.0ng/ml) prepared in antigen incubation buffer and also prepared in the appropriate dilution of plasma.

An in-dwelling Viggo secalon universal central venous catheter of 1.2 mm i.d. with a secalon universal flow-switch was placed in the right jugular vein of each animal on the first day of the study and whenever necessary, was kept patent by flushing it with heparinised normal saline (25IU/ml). This catheter was removed upon the completion of the study.

hGH was administered at 34.2ug/kg (0.1 IU/kg) via the subcutaneous route and at 307.5ug/kg (0.9 IU/kg) via the nasal route. Three sheep were used in each experiment:

(1) Subcutaneous administration of hGH as an aqueous solution prepared at 1.37mg/ml (4 IU/ml).

(2) Intranasal administration of hGH as an aqueous solution prepared at 17.57 mg/ml (51.43 IU/ml). A sheep of 40 kg would thus receive 0.35 ml of the formulation in each nostril (0.70 ml total).

(3) Intranasal administration of hGH in combination with starch microspheres (2.5 mg/kg) and LPC (0.20 mg/kg) as a lyophilised powder. To prepare the formulation, 500 mg of Spherex were dispersed in 30 ml of sterile distilled water containing 61.5 mg hGH (180 IU) and 40 mg LPC, mixed for 1h, and then freeze-dried.

For intranasal administration of solutions, a blue-line umbilical cannular of 35 cm length was inserted into the nostril of the sheep to a preset depth of 10 cm before the delivery of the solution from a 1 ml syringe. For intranasal administration of powdered formulation, a BOC endotracheal tube (red rubber, cuffed) of 6.5 mm was loaded with the powder formulation and then inserted into the nostril of the sheep to a preset depth of 6 cm before blowing the powder into the nasal cavity.

For the intranasal studies, it is necessary to sedate the sheep by use of an i.v. dose of Ketamine hydrochloride at 2 mg/kg. This is intended as a counter-

References

1. Hussain, A., Hirai, S. and Bawarshi, R., Nasal absorption of natural contraceptive steroids in rats - progesterone absorption, J. Pharm. Sci. 70, 1981, 466-467.

2. Hussain, A., Hirai, S. and Bawarshi, R., Nasal absorption of propranolol from different dosage forms by rats and dogs, J. Pharm. Sci. 69, 1980. 1411-1413.

3. Salzman, R., Manson, J.E., Griffing, C.T., Kimmerle, R., Ruderman, N., McCall, A., Stoltz, E.I., Mullin, C., Small, D., Armstrong, J. and Melly, J.S., Intranasal aerosolized insulin, N. Eng. J. Med. 312, 1985, 1078-1084.

4. Chien, Y.W., and Chang, S.F., Intranasal Dry Delivery for Systemic Medications CRC Critical Reviews in Therapeutic Drug Carrier Systems 4, 67ug (1987).

5. Hanson, M., Gazdick, G., Cahill, J. and Augustine, M., Intranasal delivery of the peptide, salmon calcitonin, in S.S. Davis, L. Illum and E. Tomlinson: Advanced Delivery Systems for Peptides and Proteins, Plenum Press, London, 1986, pp. 233-242.

6. Gordon, G.F., Moses, A.C., Silver, R.D., Flier, G.F. and Carey, E., Nasal absorption of insulin: enhancement by hydrophobic bile salts, Proc. Natl. Acad. Sci. USA 82, 1985, 7419-7423.

7. Illum, L., Jorgenson, H., Bisgaard, H., Krogsgaard, O., and Rossing N., Bioadhesive microspheres as a potential nasal drug delivery system. Int. J. Pharmaceut. 39, 189-199 (1987).

8. Nagai, T., Nishimoto, Y., Nambu, N., Suzuki, Y. and Sekine, K., Powder dosage form of insulin for nasal administration, J. Control, Rel. 1, 1984, 15-22.

CLAIMS

1. A drug delivery system including a plurality of microsphere particles containing an active drug and including a surfactant material associated with each particle which surfactant material has the property of enhancing the uptake of the active drug.

2. A drug delivery system as claimed in Claim 1 comprising a transmucosal delivery system consisting of microspheres that gel in contact with the mucosal surface and contain a pharmacological agent and an adjuvant in the form of an absorption enhancer, a mucolytic agent or an enzyme inhibitor, either separately or together.

3. A drug delivery system as claimed in Claim 1 or Claim 2 in which the microspheres are starch microspheres of a size between 10 and 100 um administered intranasally together with an absorption enhancer to improve the bioavailability of drugs.

4. A drug delivery system as claimed in Claim 1 for intranasal administration where an active pharmacological agent and a pharmaceutical adjuvant (in the form of an absorption enhancer, mucolytic agent or enzyme inhibitor) is incorporated during the process of microsphere preparation.

5. A drug delivery system as claimed in Claim 4 for nasal administration but where the pharmacological agent and adjuvant are sorbed into or onto the microspheres after preparation and then freeze dried if necessary.

6. A drug delivery system as claimed in Claim 1 or Claim 2 wherein the microspheres are prepared from starch derivatives, gelatin, albumin, collagen, dextran, dextran derivatives or similar materials.

7. A drug delivery system as claimed in Claim 3 wherein the microspheres are modified by a process of crosslinking.

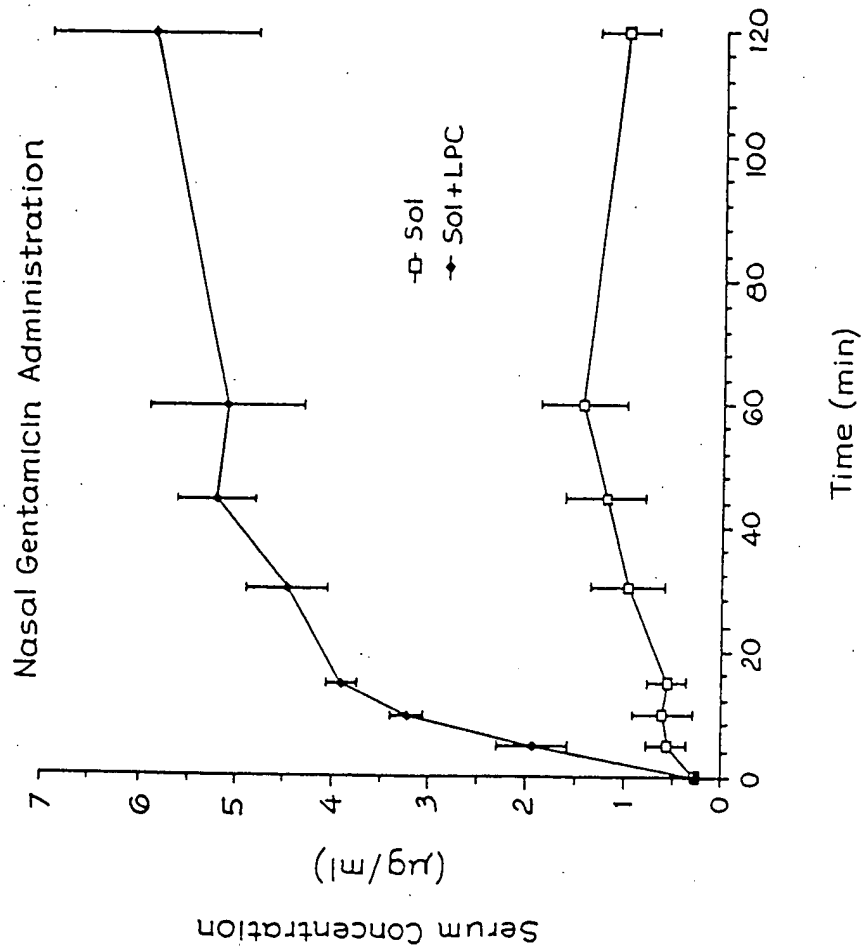
Nasal Gentamicin Administration
Sol
Sol+LPC
Serum Concentration
(µg/ml)
Time (min)
0
1
2
3
4
5
6
7
0
20
40
60
80
100
120

Fig. 1

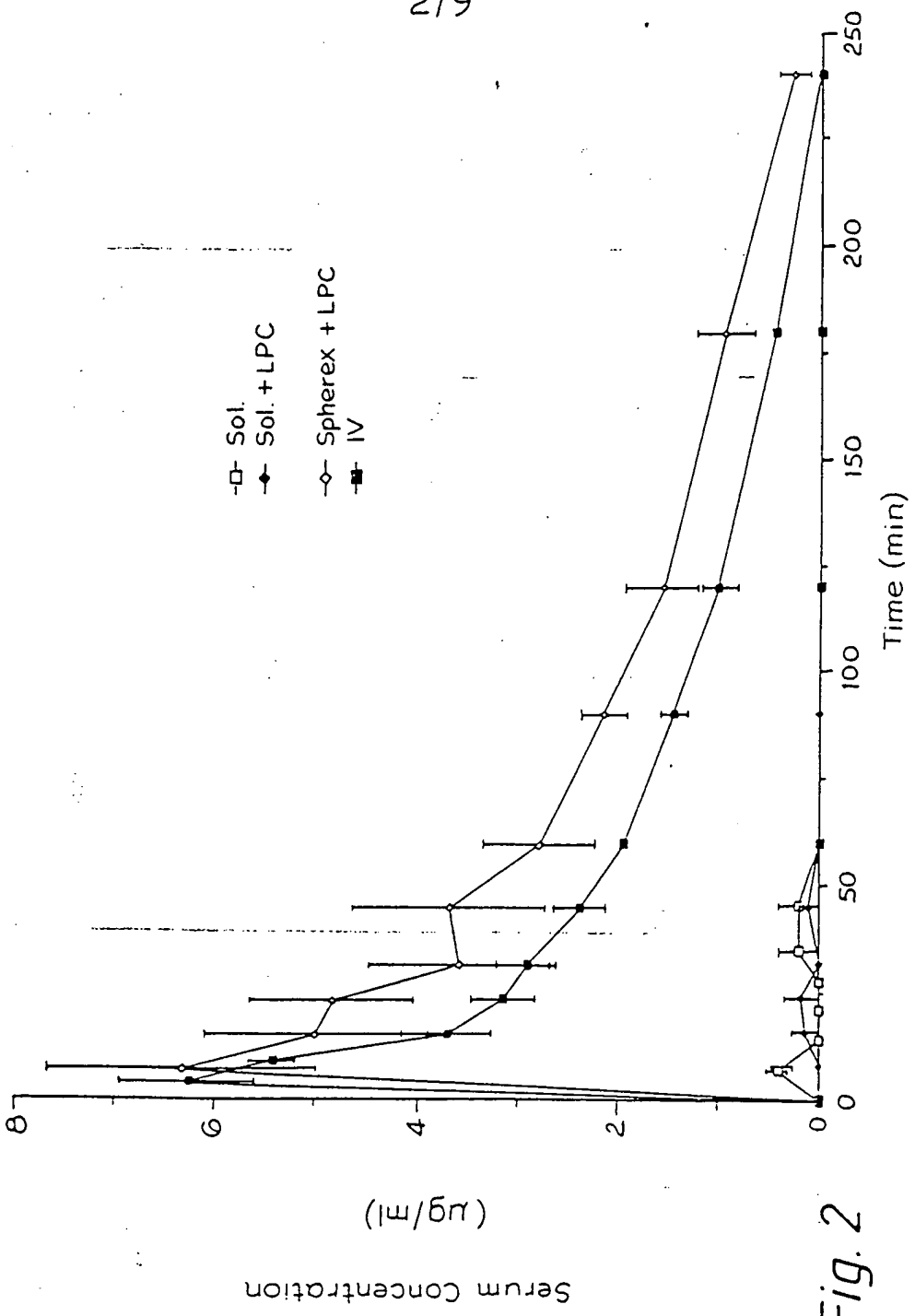
Sol.
Sol. + LPC
Spherex + LPC
IV
Serum Concentration
(µg/ml)
0
2
4
6
8
0
50
100
150
200
250
Time (min)

Fig. 2

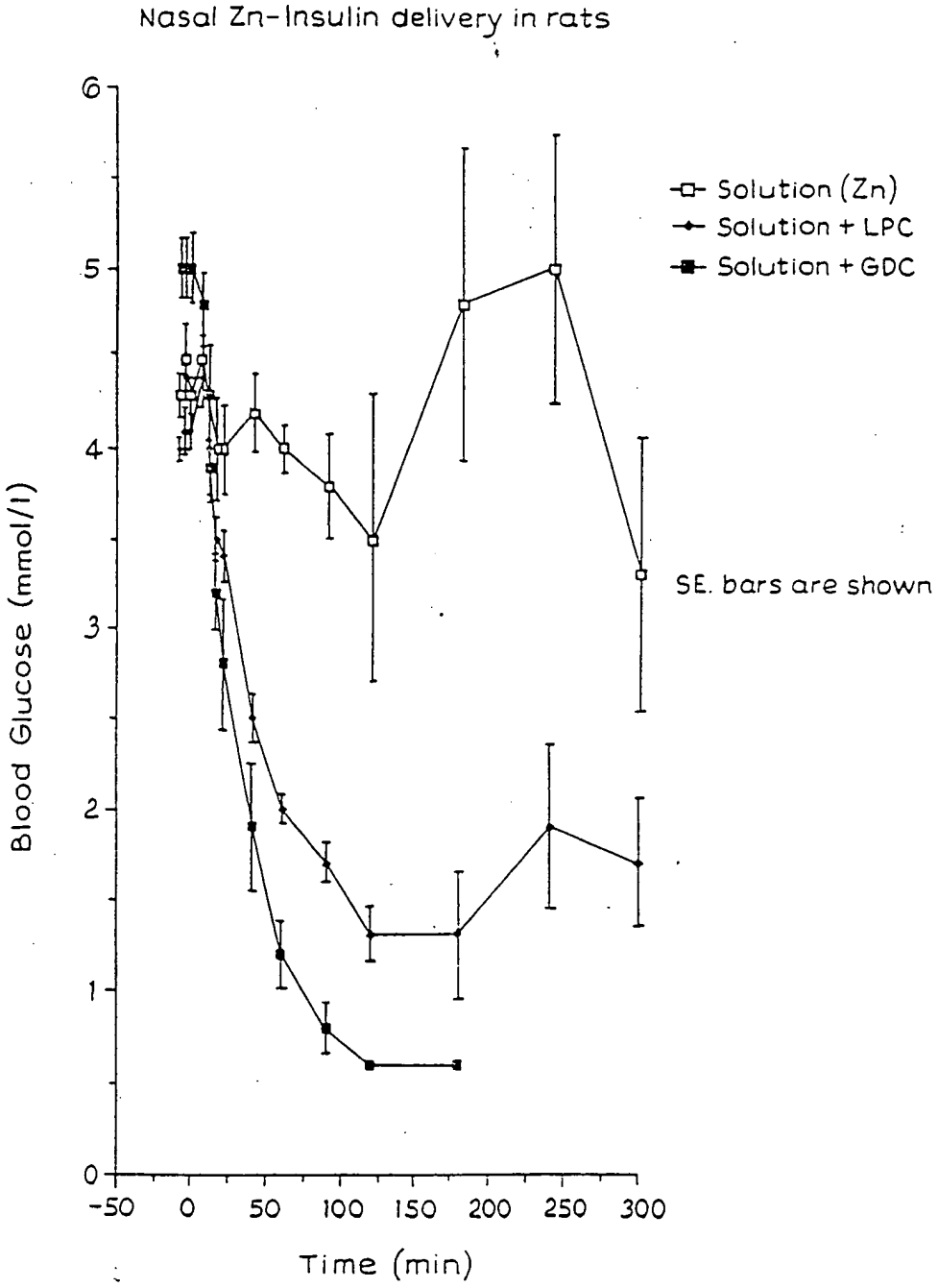
Nasal Zn-Insulin delivery in rats
Blood Glucose (mmol/l)
Time (min)
Solution (Zn)
Solution + LPC
Solution + GDC
SE. bars are shown
0
1
2
3
4
5
6
-50
0
50
100
150
200
250
300

Fig. 3

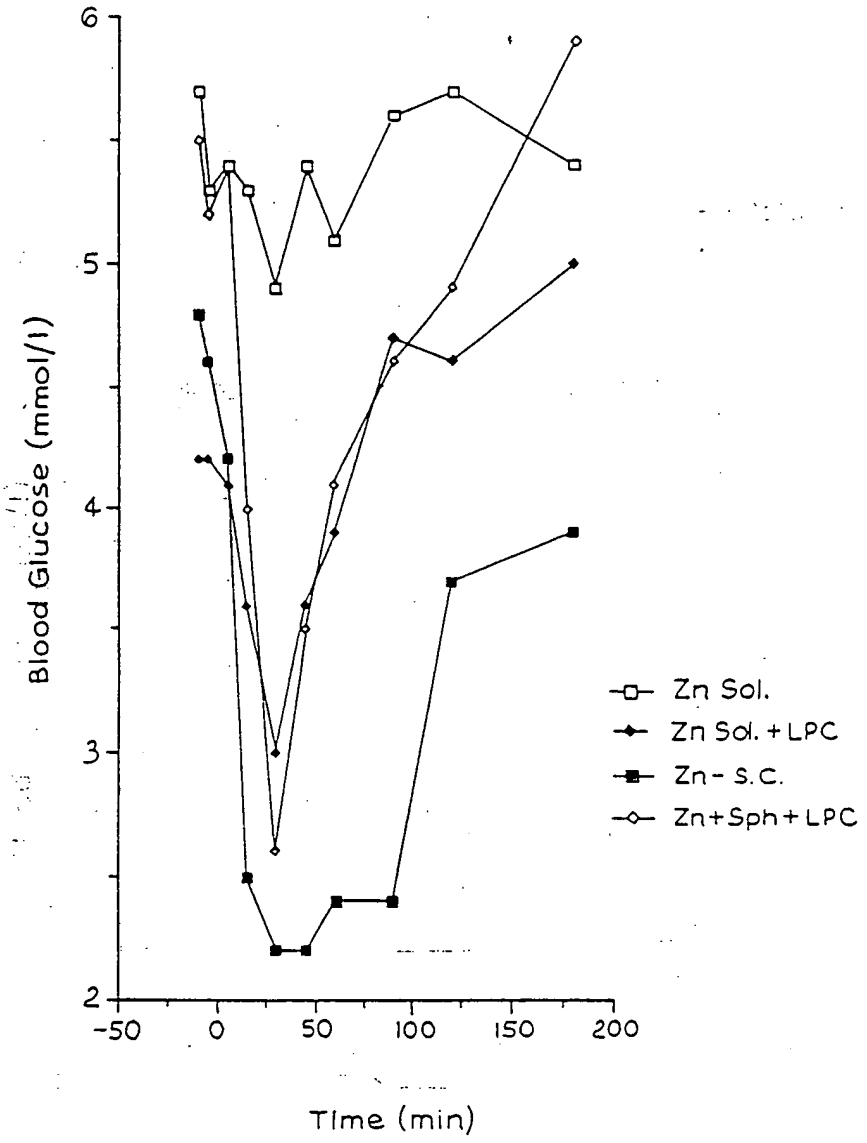
Blood Glucose (mmol/l)
6
5
4
3
2
-50
0
50
100
150
200
Time (min)
Zn Sol.
Zn Sol. + LPC
Zn - S.C.
Zn + Sph + LPC

Fig. 4

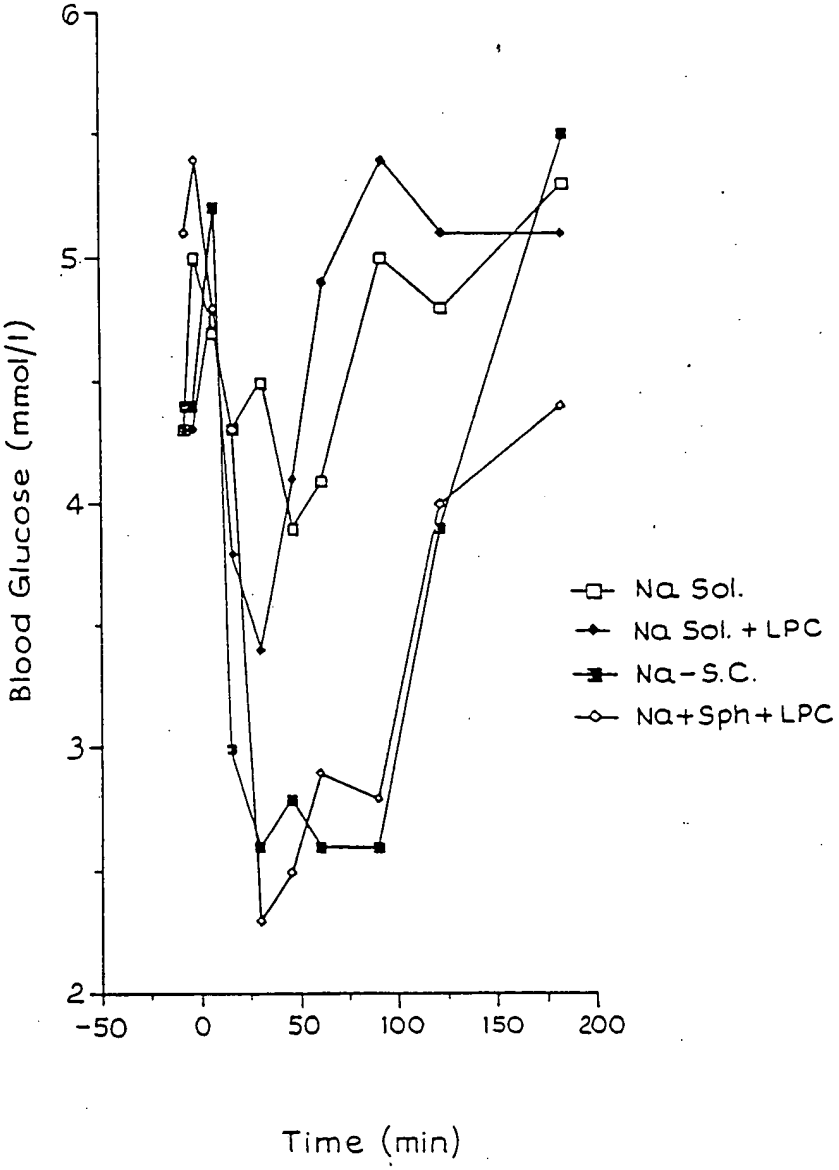
6
5
4
3
2
Blood Glucose (mmol/l)
-50
0
50
100
150
200
Time (min)
Na Sol.
Na Sol. + LPC
Na-S.C.
Na+Sph+LPC

Fig. 5

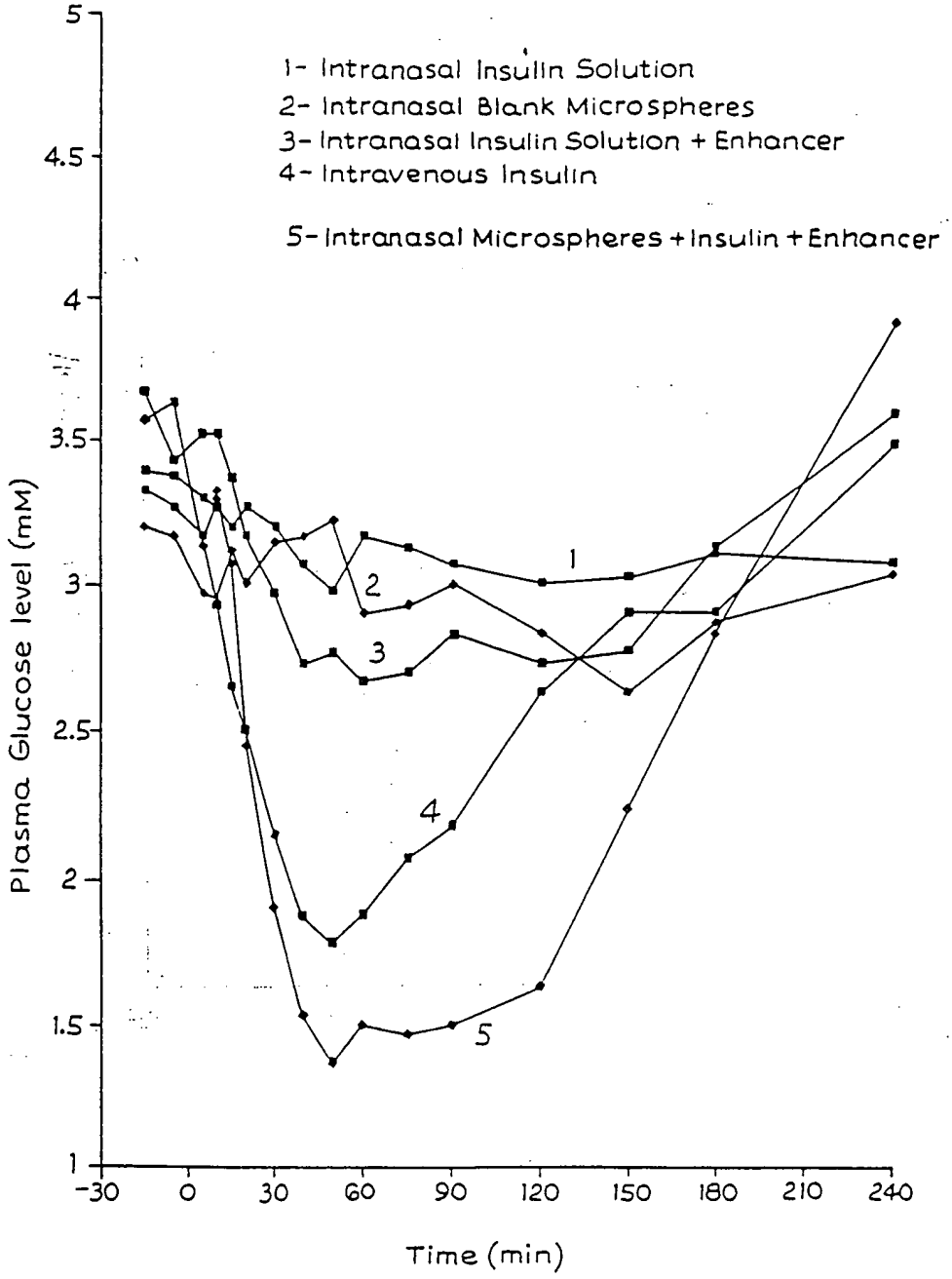
1- Intranasal Insulin Solution
2- Intranasal Blank Microspheres
3- Intranasal Insulin Solution + Enhancer
4- Intravenous Insulin
5- Intranasal Microspheres + Insulin + Enhancer
Plasma Glucose level (mM)
Time (min)
5
4.5
4
3.5
3
2.5
2
1.5
1
-30
0
30
60
90
120
150
180
210
240

Fig. 6

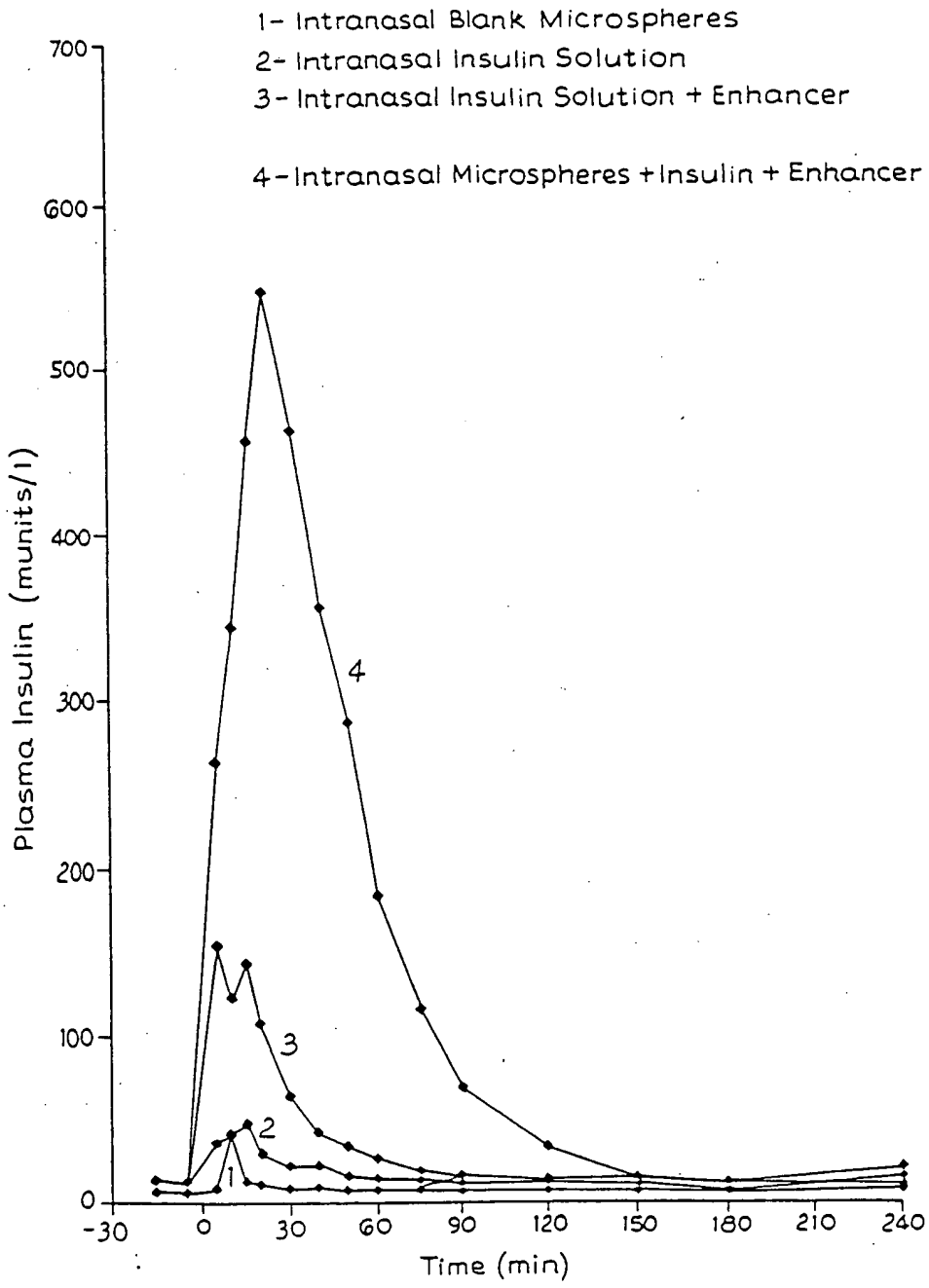
1- Intranasal Blank Microspheres
2- Intranasal Insulin Solution
3- Intranasal Insulin Solution + Enhancer
4- Intranasal Microspheres + Insulin + Enhancer
Plasma Insulin (munits/l)
700
600
500
400
300
200
100
0
-30
0
30
60
90
120
150
180
210
240
Time (min)
1
2
3
4

Fig. 7

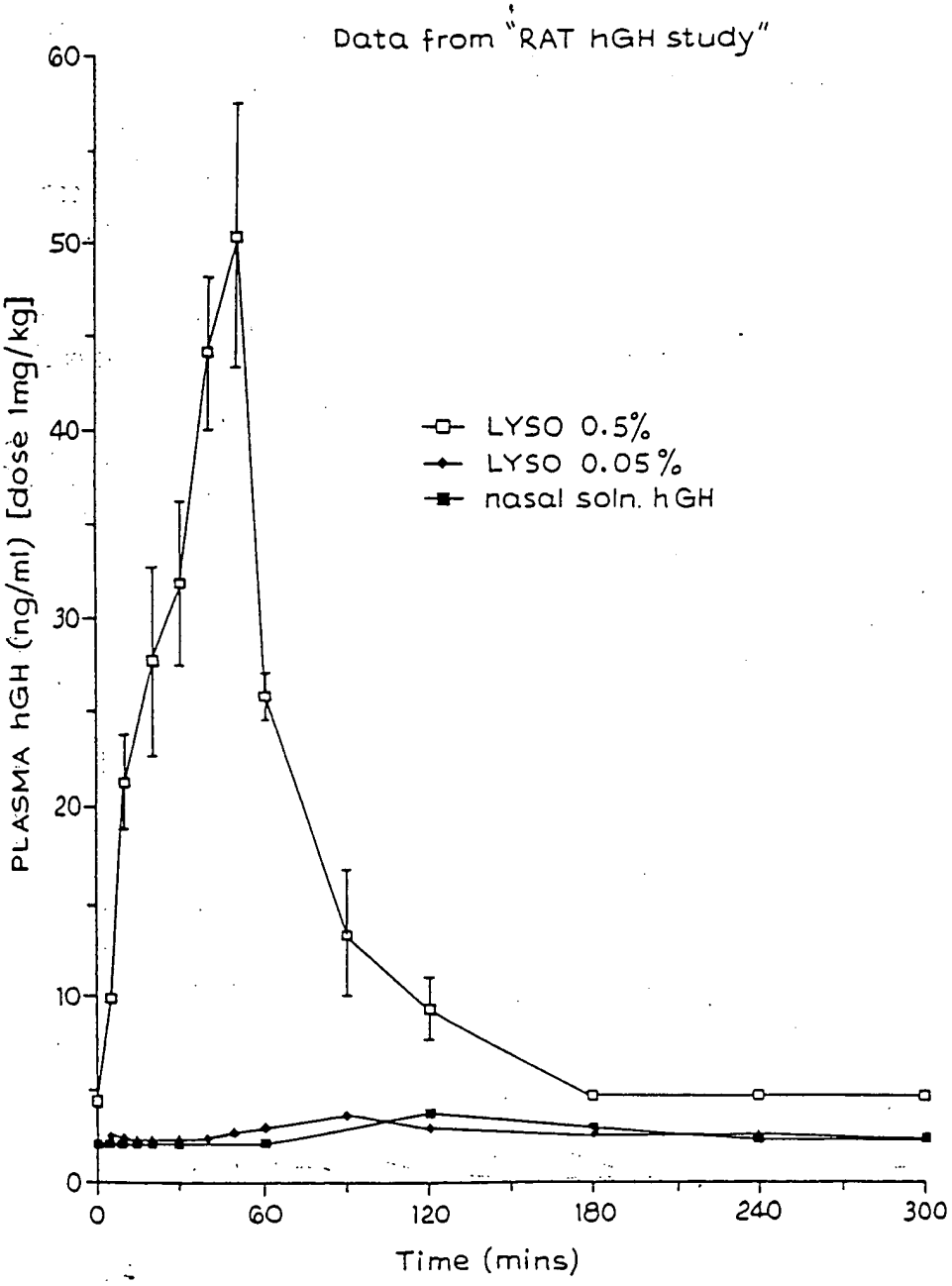
Data from "RAT hGH study"
PLASMA hGH (ng/ml) [dose 1mg/kg]
60
50
40
30
20
10
0
0
60
120
180
240
300
Time (mins)
LYSO 0.5%
LYSO 0.05%
nasal soln. hGH

Fig. 8

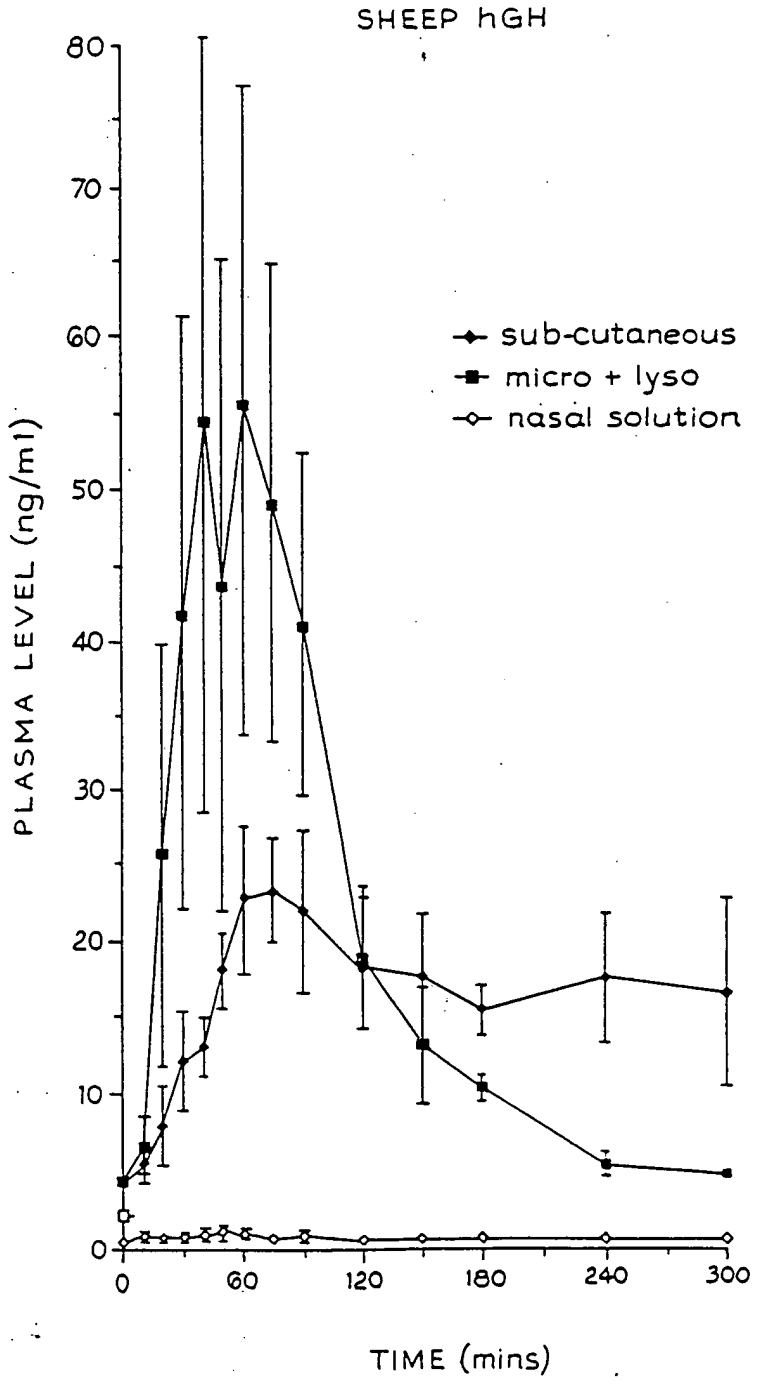
SHEEP hGH
PLASMA LEVEL (ng/ml)
TIME (mins)
sub-cutaneous
micro + lyso
nasal solution
0
10
20
30
40
50
60
70
80
0
60
120
180
240
300

Fig. 9

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 88/00396

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: A 61 K 9/16; A 61 K 9/72

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
P,X	EP, A,0257915 (INNOVATA BIOMED LTD) 2 March 1988 see the whole document	1,2,4,5. 10
X	Journal of Pharmaceutical Sciences, volume 72, no. 9, September 1983, American Pharmaceutical Association, L. Illum et al.: "Effect of the nonionic surfactant poloxamer 338 on the fate and deposition of polystyrene microspheres following intravenous administration", pages 1086-1089 see the whole article	1
P,Y	Chemical Abstracts, volume 107, no. 20, 16 November 1987, (Columbus, Ohio, US), L. Illum: "Microspheres as a potential controlled release nasal drug delivery system", see page 488, abstract 183492g,	1-18
Y	& NATO ASI Ser., Ser. A 1986, 125 (Delivery Syst. Pept. Drugs), 205-10	

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
19th August 1988	19. 09. 88

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	P.C.G. VAN DER PUTTEN

Form PCT/ISA/210 (second sheet) (January 1985)

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 8800396 SA 22248

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 09/09/88 The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0257915	02-03-88	WO-A- 8801165	25-02-88
		AU-A- 7754987	08-03-88
GB-A- 2176105	17-12-86	JP-A- 61282320	12-12-86
		AU-A- 5824786	11-12-86
		FR-A- 2582511	05-12-86
		DE-A- 3617728	04-12-86
		BE-A- 904863	03-12-86
		SE-A- 8602530	05-12-86
		NL-A- 8601285	02-01-87
		LU-A- 86457	13-01-87
FR-A- 2081353	03-12-71	NL-A- 7018033	15-06-71
		DE-A- 1962496	24-06-71
		US-A- 3681500	01-08-72
		GB-A- 1333767	17-10-73
		CA-A- 942671	26-02-74
		BE-A- 760251	11-06-71
EP-A- 0122036	17-10-84	JP-A- 59163313	14-09-84
		US-A- 4613500	23-09-86